

Supplemental Figures

Table S1. Hsp90 client kinases harbor more and more harmful genetic variation across humans.

	p-value	Rho (Pearson's)
PIC ¹ nucleotide diversity ~ PIC HIS ²	0.04965	0.157
PIC PolyPhen probable ~ PIC HIS	0.0099	0.206
PIC GERP ≥ 5 ~ PIC HIS	0.7593	0.02473

¹Phylogenetic independent contrasts

²HIS- Hsp90 interaction score

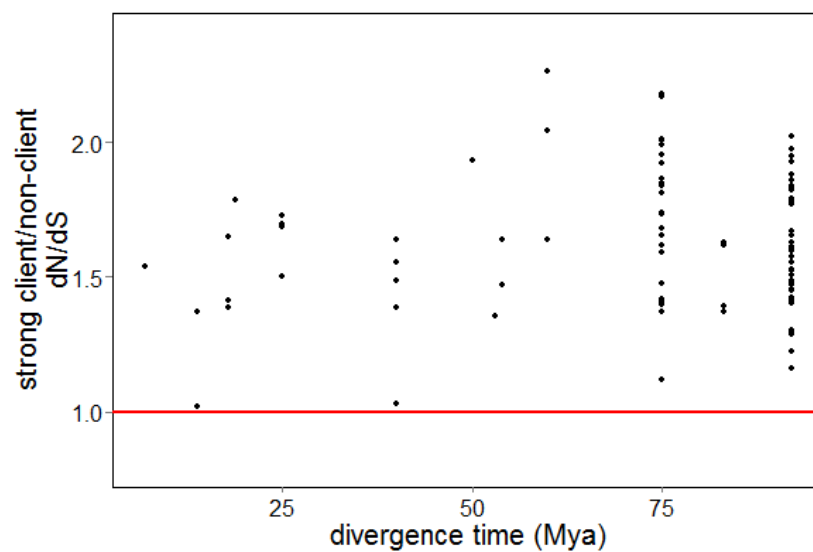


Figure S1. The average strong client kinase dN/dS was always greater than the non-client kinase dN/dS across mammals, regardless of species used for pairwise dN/dS comparison (ratios were always greater than 1, red line).

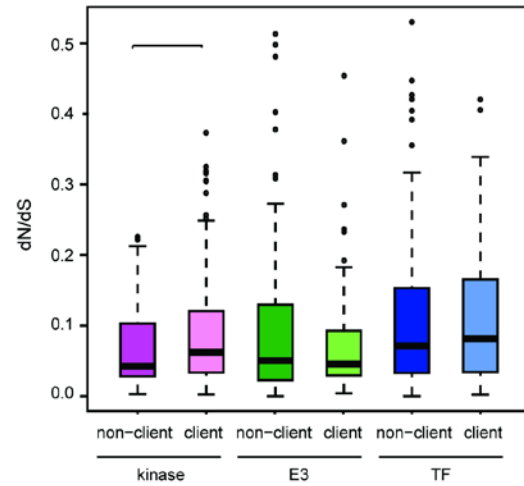


Figure S2. E3 and TF clients do not evolve faster than their respective non-clients. In contrast to kinases ($p = 0.01221$, Wilcoxon rank-sum test), transcription factor and E3 ligase clients do not show significantly greater dN/dS than transcription factors and E3 ligase non-clients.

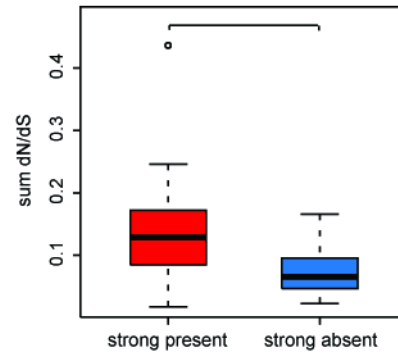


Figure S3. Hsp90 client status is associated with divergence of gene duplicates. Divergence was measured as the sum dN/dS for each gene duplicate pair. Pairs of gene duplicates that encode at least one strong Hsp90 client (n=19) diverge faster than pairs without an Hsp90 client (n=27) ($p = 0.001183$, Wilcoxon rank-sum test).

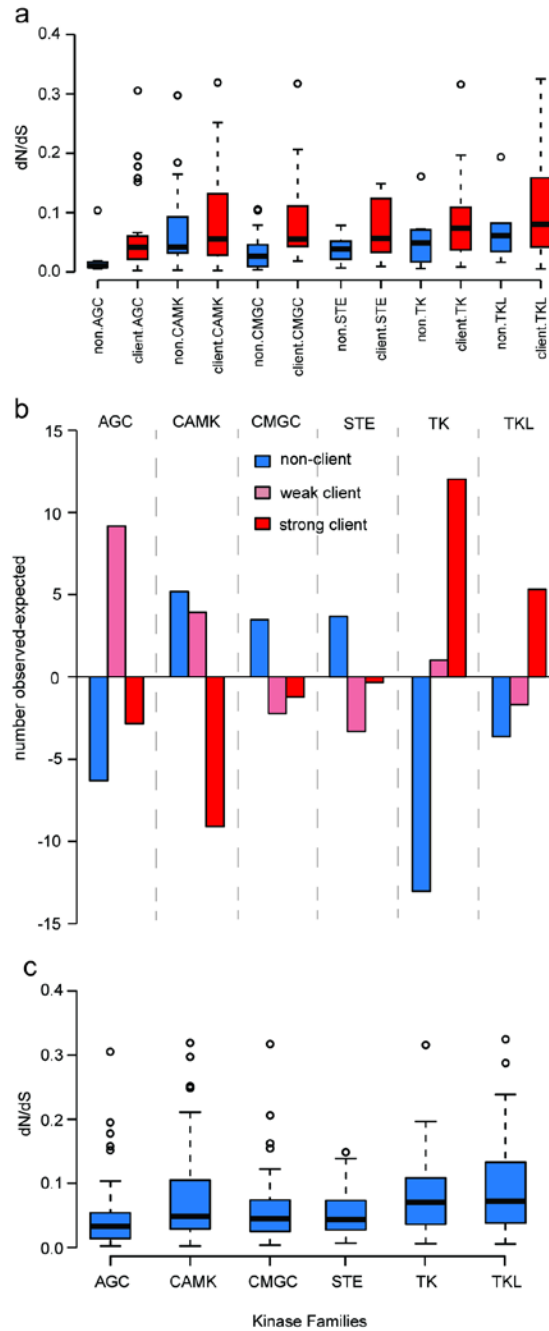


Figure S4. Hsp90's effect on dN/dS is observed in all kinase groups. a) In each kinase group, the Hsp90 clients tended to show greater dN/dS than non-clients. b) The TK and TKL groups were enriched for strong Hsp90 clients; the CAMK group was most depleted for strong Hsp90 clients. The expected number of non-clients, weak clients, and strong clients was calculated by assuming an equal distribution of all client states across all kinase groups. c) The TK and TKL

families show the largest median values of dN/dS, albeit their dN/dS did not significantly differ from the other kinase groups.

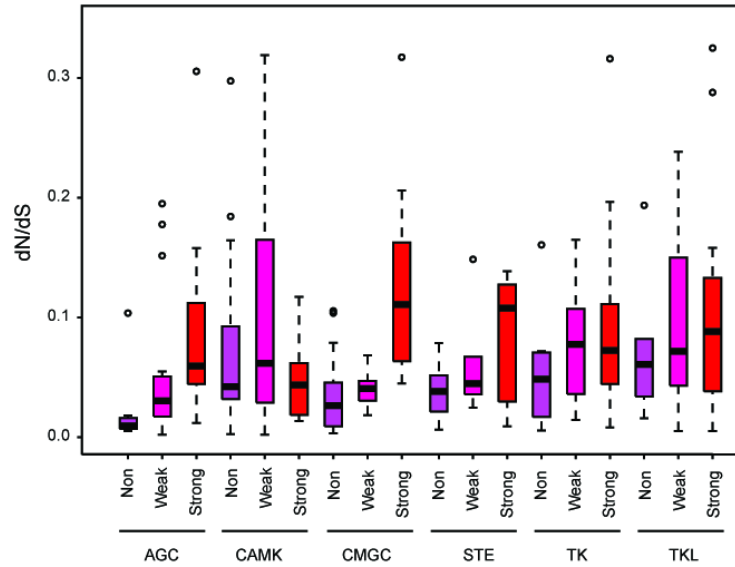


Figure S5. Family-specific differences for the association of Hsp90 client status and dN/dS.

In general, strong kinase clients (red) tended to have greater dN/dS than weak kinase clients (pink), and weak kinase clients (purple) tended to have greater dN/dS than kinase non-clients. The TK and CAMK groups did not follow this pattern, with weak kinase clients tending to have greater dN/dS, presumably due the broad distribution of dN/dS for weak kinases in these groups.

Supplementary Text

Interaction with Hsp90 is associated with faster evolutionary rates in clients, but not co-chaperones or otherwise collaborating proteins.

We failed to detect an effect of Hsp90 client status on the evolutionary rate of transcription factors and E3 ligases (Figure S2). In contrast to the tested kinases, which evolved from a common ancestor, transcription factors and E3 ligases are not monophyletic (Li, et al. 2008; Vaquerizas, et al. 2009) and hence likely differ greater in other features influencing evolutionary rate. Hence, we subdivided the tested transcription factors (Vaquerizas, et al. 2009) and E3 ligases (Li, et al. 2008) into phylogenetically related groups for further tests.

For transcription factors, this subdivision approach was hampered by the extremely low number of transcription factors that interact with Hsp90 (58 out of 843 tested) (Taipale, et al. 2012). Comparing evolutionary rates of clients and non-clients in phylogenetically related groups was not feasible (average group size 18.4, average number of clients 1.8, and non-clients 16.7).

For E3 ligases, we used previously described subdivisions and tested for association of Hsp90 client status and dN/dS. Specifically, we divided the E3 ligases into RING finger domain-containing (RNF) and non-RNF proteins (Li, et al. 2008). We found no significant effect of Hsp90 client status on RNF and non-RNF E3 ligases. Similarly, testing only E3 ligases with a Kelch fold (Taipale, et al. 2012) yielded no evidence that Hsp90 interactors evolved faster than non-interactors. E3 ligases also contain small domains with roles in recognition of ubiquitinated substrates. We attempted to cluster the tested E3s by these domains as identified in Pfam, using several different thresholds for domain identity. Again, we found no significant differences in dN/dS associated with Hsp90 interaction in these groups.

This persistent failure in light of the clear and consistent signal in kinases prompted us to further explore the literature on Hsp90 and E3 ligase interaction. In contrast to TFs and kinases, which require Hsp90's assistance to reach their mature fold, E3 ligases may interact physically with Hsp90 for another reason. Indeed, some E3 ligases have been shown to collaborate with Hsp90 in the degradation of other proteins, rather than being chaperoned by Hsp90 (Murata et al. 2001; Giannini & Bijlmakers 2004; Morishima et al. 2008). If E3 ligases generally are Hsp90-

collaborating proteins, we would not expect an Hsp90-associated effect on E3 ligase dN/dS, akin to prior observations with other Hsp90-associated proteins such as Hsp70 and various Hsp90 co-chaperones, all of which are rather highly conserved across eukaryotes.